PRINCIPLES OF PHASE MICROSCOPY

Phase microscopy puts in the hands of the microscopist a unique technique of immeasurable value in the examination of the structural detail of living, transparent organisms and other specimens of similar optical properties. So significant is its importance that Prof. F. Zernike of Holland was awarded the Nobel Prize in 1953 for his pioneering work in phase microscopy.

Stated in simplest terms, the function of the phase microscope is the conversion, through optical manipulation, of "optical path" differences into visible differences of light intensity. (Optical path being the product of refractive index times thickness.) The American Optical Company was among the first to recognize the virtually limitless application of phase microscopy and, after long and intensive development effort, introduced its initial phase microscopes in 1947.

In conventional microscopy, the details within the specimen are either darker or lighter than one another, in most cases as a result of differential staining. The darker the detail, the greater the amount of light absorbed as it passes through the plane of the specimen. In this manner, each structural element acts as an absorbing medium and weakens, to a greater or lesser degree, the light wave as it passes through. This reduction in intensity results in a corresponding reduction in light wave amplitude (distance from crest to trough of the wave) as shown in *figure A*. The selective absorption described here produces a readily visible image because specimen detail appears as differences in brightness or color to which the eye is sensitive.

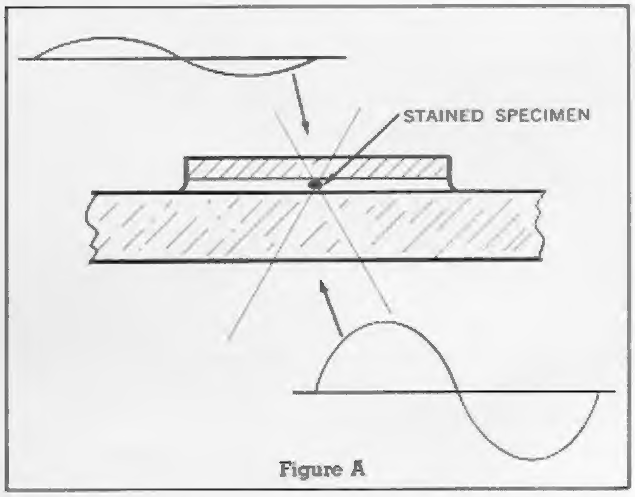


Figure A

The *phase specimen* (e.g. an unstained, small, living organism) is transparent, colorless and, typically, is mounted in a transparent media of almost identical optical properties. Because of the transparent nature of the fine detail within the phase specimen, the amplitude of the light waves passing through the specimen and its surrounds is not appreciably altered. With very little or no light absorption by the structural elements of the specimen, the intensity of the light waves remains constant. Thus, because varying degrees of light intensity are lacking, an image visible to the eye cannot be formed in the classical manner.

However, as light passes through the phase specimen, another most significant phenomenon occurs, called diffraction. There exists within the fine structural detail of the phase specimen, and the specimen as a whole relative to its surround, what is termed "optical path" differences resulting from differences in index of refraction and thickness. Differences in optical path give rise to diffraction by the specimen detail. The more pronounced or "abrupt" the discontinuities in optical path the greater the diffraction effect.

One result of diffraction is that light is scattered as compared to the undeviated light which is transmitted directly through those areas of the specimen where there is insufficient detail to cause diffraction. The second important characteristic of diffraction is that there is a difference in *phase* between the diffracted and undeviated or direct light. The phase microscope makes it possible to convert differences in optical path to which the eye is insensitive into amplitude or intensity differences which the eye can see (without the use of staining).

The phase microscope utilizes two unique components: (1) an annular diaphragm below the condenser which directs a hollow cone of light to the transparent specimen; and (2) a conventional microscope objective which is modified by the addition of a diffraction or "phase" plate. This plate is constructed and positioned in a manner which separates the diffracted and direct light coming from the specimen and alters their intensity and phase relationships so that they combine in the image plane of the eyepiece to form a visible image.

As the light passes through the phase specimen and its surround, diffraction occurs at each point where internal structural detail (of different optical path) is

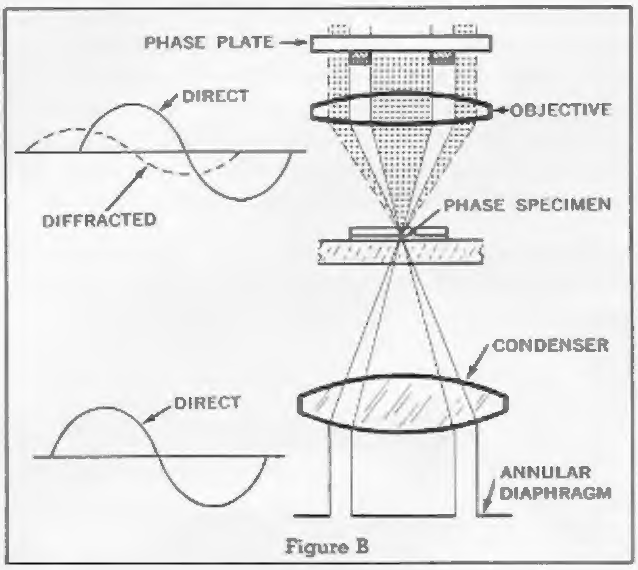


Figure B

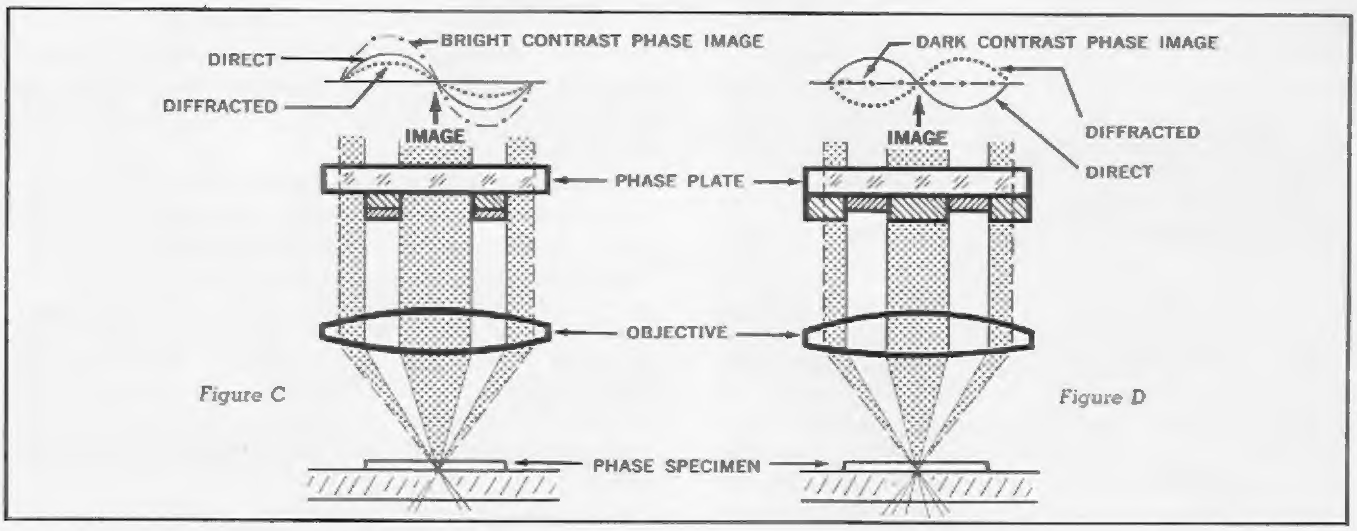


Figure C

Figure D

present. Similarly, the abrupt discontinuity in optical path around the edges of the specimen where it adjoins its surround causes diffraction. This diffracted light is ¼ wave length out of phase with light not diffracted by specimen detail which passes *directly* through the specimen plane. The objective lens thus receives both the direct light and that portion of the diffracted orders of light which fall within the limits of the objective aperture (See *figure B*).

We have, then, this situation, as all the light moves from the plane of the specimen toward the diffraction or "phase" plate: The direct light with considerably greater intensity than the diffracted light moves as a cone of concentrated light toward coincidence with the "ring" of the diffraction plate. The diffracted light, relatively weak in intensity and retarded in phase by ¼ wave length, moves so as to be distributed over the whole aperture of the diffraction plate.

Since the direct light is brighter, its intensity would tend to overbalance the weaker, diffracted light and dilute or destroy image contrast. To compensate and bring the direct and diffracted light into "balance," a metallic absorbing film in the form of a ring-shaped disc is utilized. This film or coating on the ring of the phase plate reduces the intensity of the direct light. Such absorption tends to "equalize" intensities or brightness. Thus, when the diffracted and direct light later combine at the eyepiece focal plane, they are balanced to achieve the desired degree of contrast.

Simultaneously, with the absorption procedure described above, the diffraction plate performs a second important function. Utilizing a phase retarding material, the relative phase relationship of the diffracted and direct light is altered by ¼ wave length. If a phase retarding material is placed upon the ring-shaped disc of the diffraction plate, a "Bright Contrast" image results. In this case, as shown in *figure C*, the direct light is retarded by ¼ wave length, bringing it *into phase* with the diffracted light. In this manner, the diffracted light wave is reinforced by the "additive" superposition of the direct light wave. The result is a specimen image which appears bright against a darker background. (This assumes the specimen has greater optical path characteristics than its surround as is generally the case.)

When the phase retarding material is placed upon all areas of the diffraction plate other than the ring-shaped disc, a "Dark Contrast" image results. As shown in *figure D*, the diffracted light is retarded by ¼ wave length. The effect of this "shift" is to bring the direct and diffracted light together at the image plane of the eyepiece ½ wave length out of phase. The result is a "subtractive" superposition of the light waves whereby the direct and diffracted waves cancel each other to form an image darker than its surround.

"B-Minus" Phase Contrast is a modification of "Dark Contrast."

This brief and non-mathematical explanation of phase contrast microscopy, with the other information presented in this reference manual, should serve as a sufficient guide for the useful application of the phase microscope. For those who wish to delve further into the theory and mathematics of phase microscopy, refer to "Phase Microscopy", J. Wiley & Sons, 605 3rd Ave., New York, N. Y., 10016.

Printed in U.S.A.

The definitive difference is PHASE*Star*

REFERENCE MANUAL

AO Series 10
Phase Microscope Equipment

AMERICAN OPTICAL COMPANY
INSTRUMENT DIVISION • BUFFALO, NEW YORK 14215

TABLE OF CONTENTS

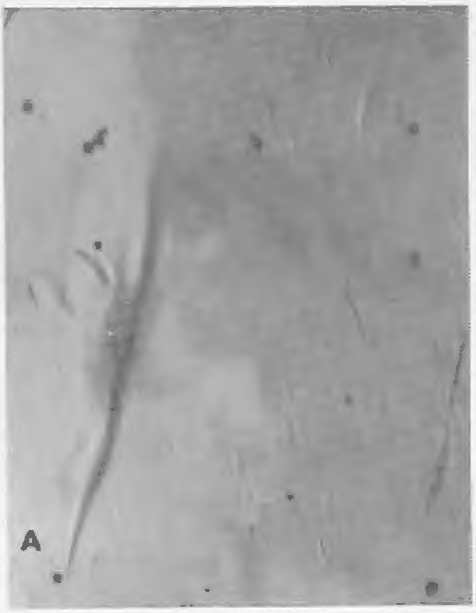
INTRODUCTION

Phase microscopy reveals details in living or unstained specimens too transparent to be seen with ordinary Brightfield microscopy. Two distinguishing components of a phase microscope are: (a) an annular diaphragm below the condenser, as shown in *figure 1;* and (b) a diffraction or "phase" plate at the rear focal plane of the objective.

AO offers three phase contrasts: Dark, Bright and B-minus. The Dark Contrast phase objectives show details of greater optical path (refractive index x thickness) darker than surrounding details of lesser optical path, as shown in *figure 2.* Bright Contrast, *figure 3,* shows the specimen bright against a darker background. B-minus, *figure 4, is a modification of* Dark Contrast and is generally used for examining slightly absorbing specimens (light pigmentation or faded colors) or inclusions within other material. With the proper choice of contrast, specimen detail may be revealed with an optical equivalent of differential staining . . . sharp boundaries are provided for measurement and adequate contrast for counting.

In addition to steps for proper set up and optical alignment procedure, this booklet contains information on all AO phase parts and accessories . . . Turret and single Condenser mounts; Condensers; Standard and Long Working Distance Phase Annuli; the Phase Aperture Viewing Unit and AO Telescope Eyepiece and illuminators. Theoretical aspects of phase microscopy are briefly described on page 15.

To conserve time in phase microscopy and increase accomplishment, we suggest that you thoroughly read these instructions now and save them for future reference.



Glass fragment mounted in balsam. (Refractive index, glass 1.52, balsam 1.54.) Illustration (A): Brightfield. Illustration (B): AO Phase Contrast.

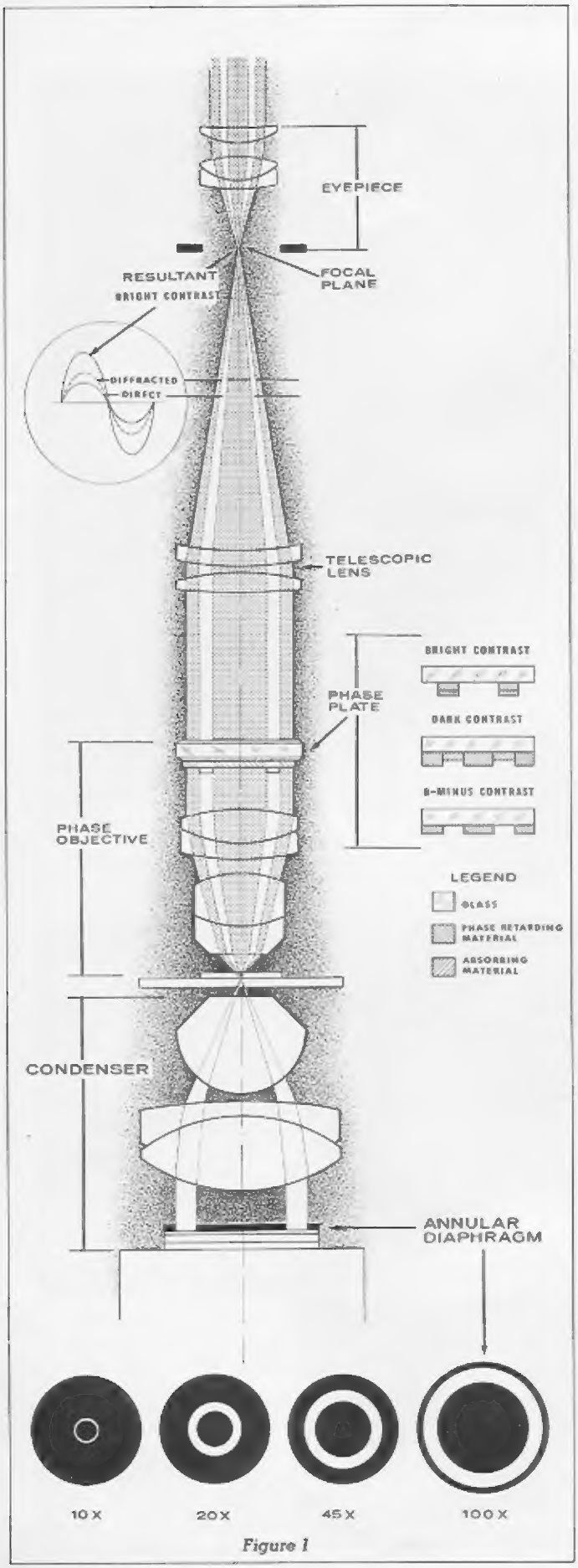


Figure 1

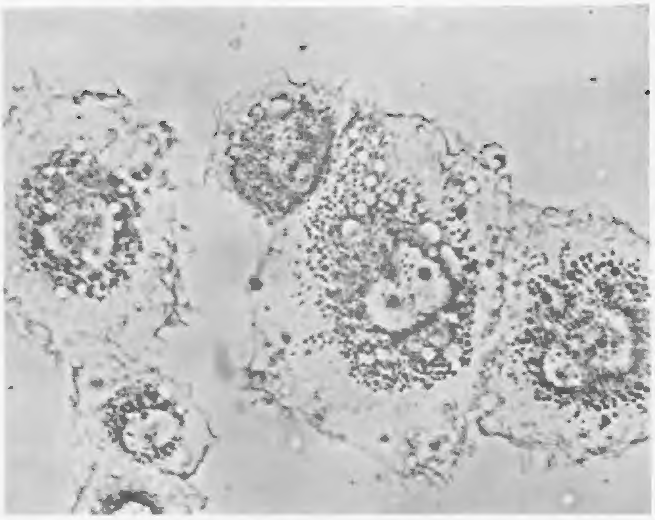


Figure 2 — Rat Carcinoma
with Dark Contrast Phase Objective

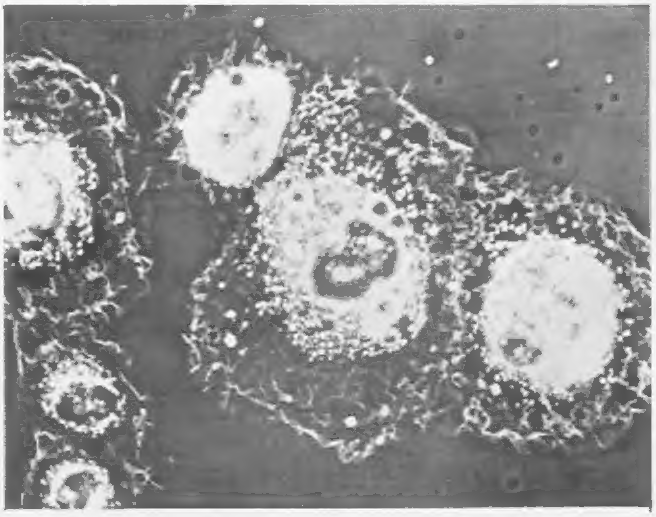


Figure 3 — Rat Carcinoma
with Bright Contrast Phase Objective

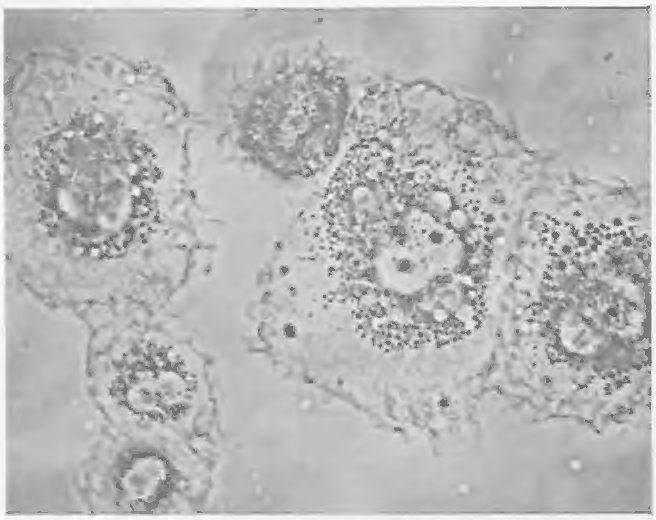


Figure 4 — Rat Carcinoma
with B-minus Contrast Phase Objective

PRELIMINARY PROCEDURE

The preliminary procedure outlined on the next four pages, while fundamental, is offered to help assure that you gain full advantage of all the advanced features built into your AO PHASESTAR, or, the phase parts and accessories which you are adding to your AO *Series 10* Microscope.

In reviewing this basic information, keep in mind that the essential adjustments of a phase microscope, stated briefly, are: (1) the field diaphragm is centered to the field of view; (2) the image of the annulus matches (superimposes on) the diffraction plate; and (3) the illumination is uniform and sufficient with the field diaphragm properly focused.

Bright Contrast	Dark Contrast	B-minus Contrast
10X, 20X, 45X, 100X	10X, 20X, 45X, 100X	100X

I. ASSEMBLING OBJECTIVES TO NOSEPIECE

When the phase objective is viewed through its back lens, the diffraction or "phase" plate may be seen as a grayish ring as shown in figure 5. The area of this ring is illuminated by the corresponding annulus in the phase condenser mount (e.g.: 10X, 16mm annulus — 10X objective).

In assembling objectives to the nosepiece, a single series of objectives (e.g.: Dark Contrast) or any combination of contrasts (Bright, Dark or B-minus) may be used. Phase objective(s) are often used in conjunction with companion AO Brightfield objectives. Regardless of the combination used or the placement sequence in the nosepiece, centration and parfocality is assured through precision tolerances held in the manufacture of AO objectives.

It is generally customary to arrange the sequence of magnification from lowest to the next highest by rotating the nosepiece clockwise. Of course, when utilizing the turret condenser, the relative position of each objective to its corresponding annulus is of primary importance.

Figure 5 — The diffraction plate appears at the rear focal plane of the phase objective as a grayish ring.

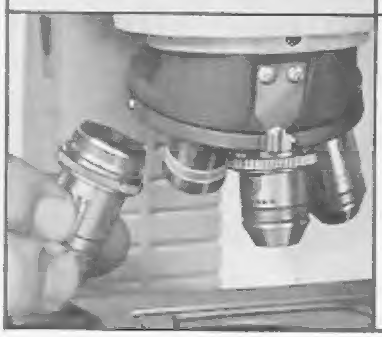
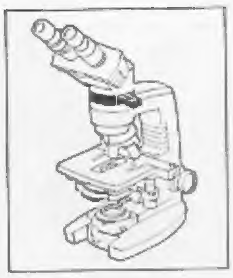
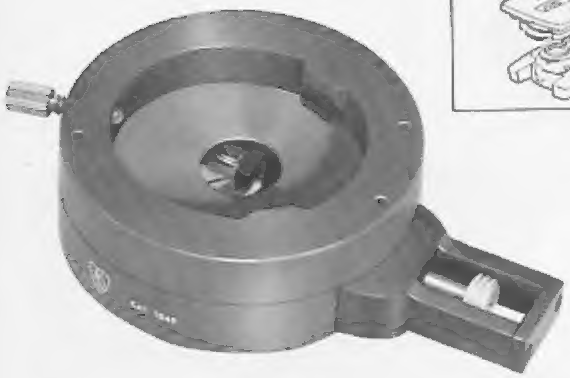


Figure 6 — Assemble objectives to the nosepiece in the same arrangement as the annular diaphragms in the Turret Condenser.





II. USE OF THE APERTURE VIEWING UNIT

The AO Phase Aperture Viewing Unit No. 1245 permits binocular viewing and focusing on the diffraction plate of each objective and the image of its corresponding annulus for fast and easy centration. The Unit — which in conjunction with the eyepiece acts as a built-in telescope — is also a convenient and rapid method of checking alignment from time to time.

The Aperture Viewing Unit is readily attached to the PHASESTAR or AO *Series 10* Microscope beneath the body and held in place with a knurled thumb screw. The operating lever can face to either side of the microscope to accommodate your technique and equipment arrangement. Regardless of orientation, the Viewing Unit will be precisely located on the optical axis of the microscope.

Figure 7 — When mounting Aperture Viewing Unit, the operating lever can be positioned on either side of microscope.

Figure 8 — Use lever to swing lens into optical path to view phase plate; swing out to view specimen.

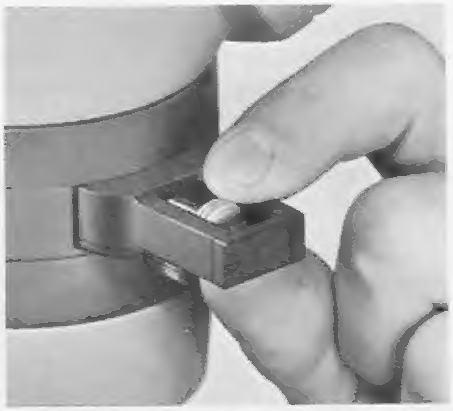
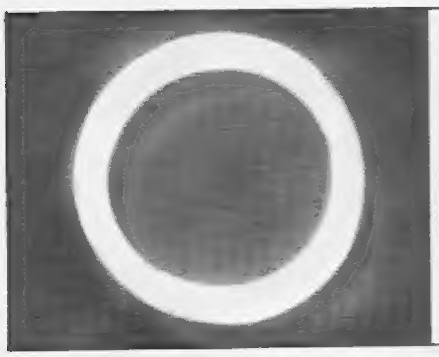


Figure 9 — Knob inside lever moves in and out to bring image of annulus and diffraction or "phase" plate into sharp focus.



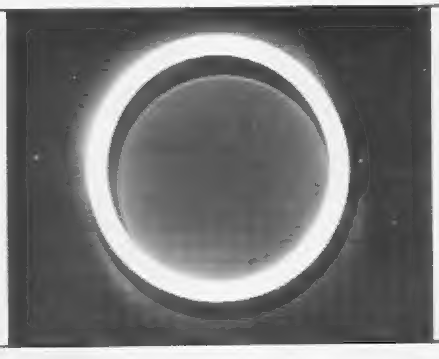


Figure 10 — Shown (on far left) the diffraction plate and the image of the annular diaphragm out of focus as they might appear when you first swing in the Aperture Viewing Unit. After adjustment using knob, they are in sharp focus as shown in the photograph to the immediate left.

AO Phase Telescope

Remove the eyepiece of the microscope and insert the Phase Telescope (AO No. 1265). By sliding the upper part in or out as required for each objective and viewing through the Telescope, you simultaneously bring the phase plate of the objective and the image of the annulus in the condenser mount into sharp focus.

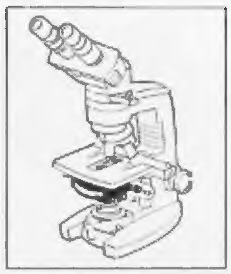
Figure 11

III. OPERATION OF TURRET CONDENSER

The Phase Turret Condenser No. 1240 contains provision for four centerable annuli and one open space, any one of which can be positioned by turning the knurled ring of the turret. Generally, the "A" setting is used to designate the 10X annulus; "B", the 20X; "C", the 45X; and "D", the 100X annulus with "O" for the open space as shown in *figure 12*. Settings may assume new meanings with other combinations of objectives and annuli.

Two captive centering wrenches are used to align each annulus to its corresponding objective. Once all objectives and annuli are properly aligned, the turret setting can be changed from one magnification, or type of contrast, to the next without further adjustment for centration being required. This is a significant feature both in terms of time saved and versatility of objective combinations.

Annuli are easily removable and interchangeable. The Standard Annuli may be used in the Turret with either the Standard or Intermediate Working Distance Condenser. The No. 1251 10X and No. 1252 20X Long Working Distance Annuli can also be used in the Turret with the Long Working Distance Condenser.



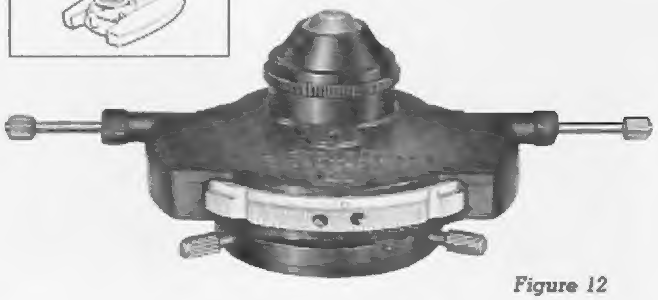


Figure 12

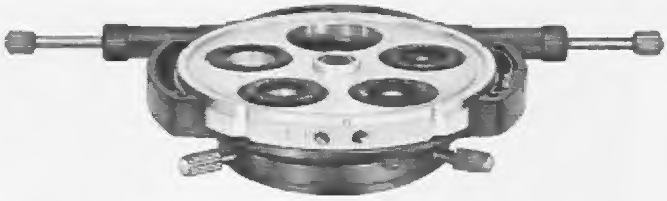


Figure 13 — In mounting the Turret Condenser to the fork, be certain that the slot in the back of the condenser assembly seats into the pin (see arrow) in the fork mount. Insert the condenser all the way into the fork; rotate until the pin engages or "snaps into" the slot; and tighten retaining screw.

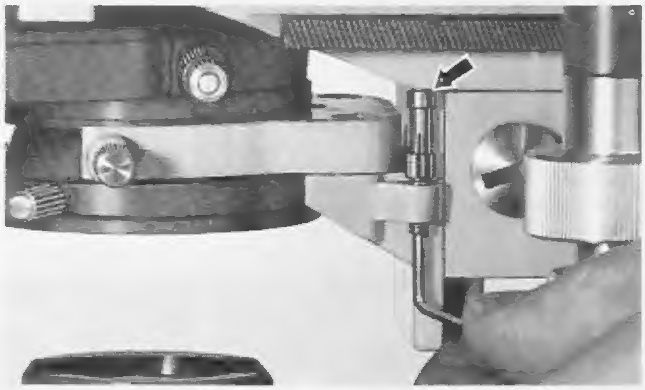


Figure 14 — The allen head stop screw with Condenser Stop Extension No. 1240-22 attached (see arrow) can be advanced or retracted, if required, to properly adjust the maximum height of the condenser in relation to stage level. If you are adding phase equipment to an AO *Series 10* Microscope, attach the Condenser Stop Extension to present stop screw in substage assembly.

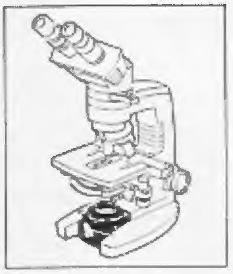
Figure 15 — Annuli are easily removed for different objective combinations. Set the turret ring to the setting for the annulus you wish to change. First, unscrew condenser; then, using spanner wrench provided, unscrew the annular diaphragm. Replacement takes only a few moments.

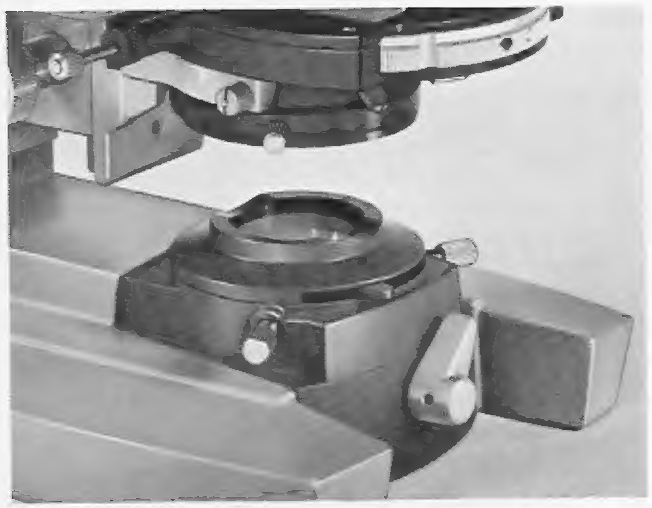
Standard Working Distance Phase Condenser

Intermediate Working Distance Phase Condenser

Long Working Distance Phase Condenser

Figure 16





1036A IN-BASE ILLUMINATOR

Figure 17 — Illuminator provides Koehler-type illumination. Two centering screws permit centration of field diaphragm. A transformer voltage setting of 6.5V is generally used. Neutral density filter, controlled by lever in front, has a transmission of approximately 10%.

IV. METHODS OF ILLUMINATION

Proper illumination for phase microscopy can be provided with either a separate or In-Base illuminator, using Koehler or Koehler-type illumination.

When using a separate light source, such as AO Model 735D, place the illuminator approximately 6" in front of the microscope. Close down the iris diaphragm of the lamp (to approx. 3/8" diameter) and direct the light to the center of the mirror (plano side). Remove any ground or diffusing glass from the illuminator. Fully close the iris diaphragm of the microscope. Focus the lamp filaments onto the condenser diaphragm leaves, adjusting plano mirror tilt as required. After filaments are in sharp focus and centered on mirror, open both diaphragms.

For further steps in using a separate illuminator, or when using built-in illuminator, follow procedure included in "Optical Alignment Procedure" beginning on the next page. With both types of illumination, a ground or diffusing glass is helpful for more even illumination. Use of ground or diffusing filters should be confined to the 10X and 20X objectives. For critical microscopy (45X and 100X), control light intensity with neutral density filters. No color filter is required with AO phase equipment; however, a green filter may be used if desired.

NO. 735D ILLUMINATOR

Figure 18 — To place the image of the lamp filaments from the mirror between and in the same plane as the filament image, partially close the iris diaphragm and focus the filament image on a wall about two feet from the lamp. Adjust the three centering screws on the back of the lamp housing until the two filament images intermesh.

NO. 645 FLUOROLUME

Figure 19 — 200 watt Mercury Arc Lamp used in combination with reflector and high speed glass condenser. Assures maximum concentration and transmission of light intensity for phase and fluorescence microscopy. Accommodates, with the use of appropriate height adapter plate, AO *Series 10* Phase Microscopes equipped with In-Base illuminator. Available with a variety of exciter, heat absorbing and neutral density filters and compact power supply.

OPTICAL ALIGNMENT PROCEDURE
For AO Series 10 Phase Microscope Equipment

Proper optical alignment procedure is of paramount importance in phase contrast microscopy. Once familiar, it can be rapidly and easily accomplished. The essential steps are outlined below. Review them carefully. It is recognized that set up techniques vary from one individual to the other; however, the basic sequence of procedure is generally the same.

Please bear in mind that centration has been carefully checked at the factory before each PHASESTAR is shipped. If you are using a recently delivered AO Phase microscope, a quick check should find centration satisfactory with but minor, if any, adjustment required.

When adding phase equipment to a *Series 10* AO microscope, the set-up procedure should be followed in detail. Also these instructions will be of value when changes are desired in type of condenser, annuli or objectives.

In this series of steps, it is assumed that you are utilizing the No. 1240 Phase Turret Condenser Mount and a Standard Working Distance Phase Condenser (rather than Intermediate or Long Working Distance) and that either Dark Contrast or Bright Contrast Phase Objectives are being employed. (The low contrast B-minus objective has no absorbing materials on the diffraction plate and is not readily seen with the telescope eyepiece or Aperture Viewing Unit.) Variations in set up for other phase accessories are considered separately.

1. Make certain that the instrument is properly set up according to "Preliminary Procedure" instructions.
2. Cleanliness is essential in phase microscopy. Check carefully to make certain that the equipment and specimen preparation is clean; in particular, the slide and cover glass, top condenser lens, and front lens of the objective.
3. When using the 1036A In-Base Illuminator, initial centration of the illuminator is most important. This is accomplished by visually aligning

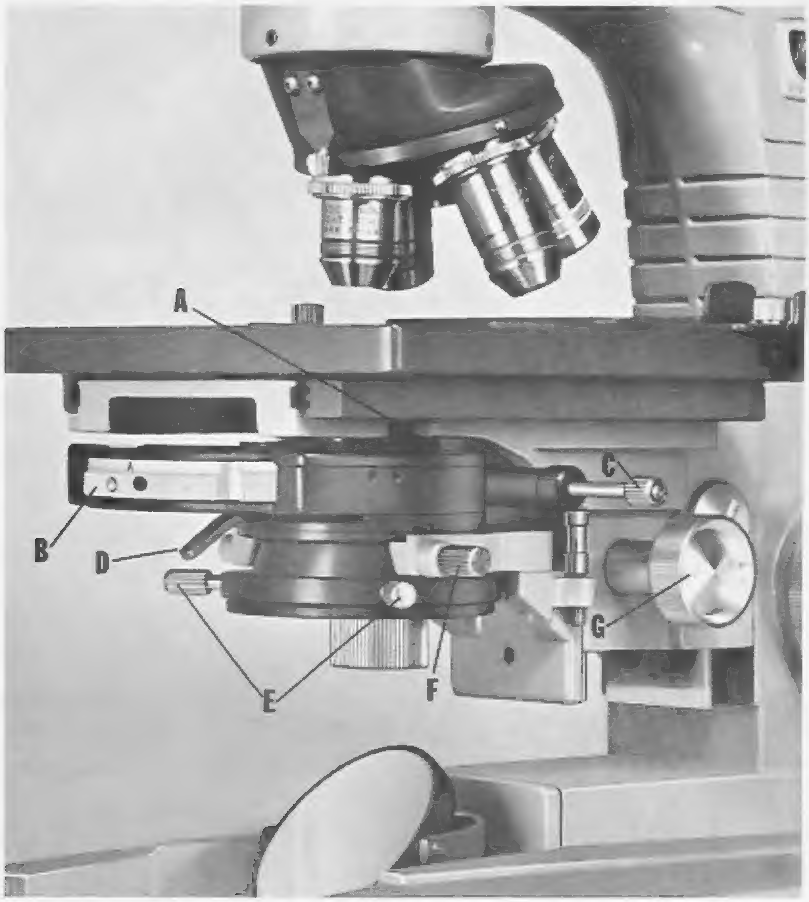


Figure 20 illustrates: (A) phase condenser; (B) rotatable knurled turret ring; (C) annular diaphragm centering wrenches; (D) turret aperture diaphragm lever; (E) centering screws for condenser; (F) retaining screw; (G) knob to raise or lower condenser and substage assembly.

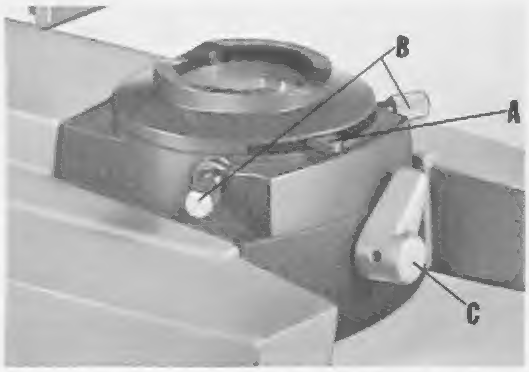


Figure 21 illustrates: (A) illuminator field diaphragm lever; (B) lamp centering screws; (C) lever to control position of neutral density filter.

Figure 22 illustrates: (A) retaining screw; (B) focusing knob; (C) lever to swing Aperture Viewing Unit into or out of optical path.

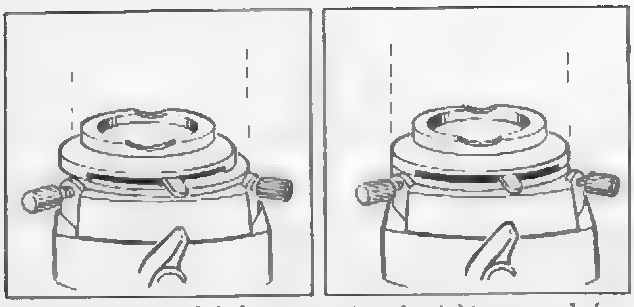


Figure 23 — Above left, lamp mount, as it might appear, before being positioned concentric with lamp housing. Above right, correctly centered.

the mount and housing of the illuminator as shown in *figure 23*. Using the two centering screws B *figure 21*, adjust the position of the mount until it is concentric with the housing. When so geometrically centered, approximately the same amount of "thread" will be exposed on both centering screws.

4. Fully open the iris diaphragms of both the illuminator, A *figure 21*, and the condenser, D *figure 20*.
5. Withdraw the two captive centering wrenches, C *figure 20*, and rotate the knurled turret ring, B *figure 20*, to the "O" setting. These wrenches *must* be withdrawn to permit turret rotation.
6. Place a stained specimen slide on the stage (as normally used in ordinary Brightfield).
7. With the extension cap in place on the stop screw of the substage assembly, raise the condenser by means of knob G, *figure 20*.

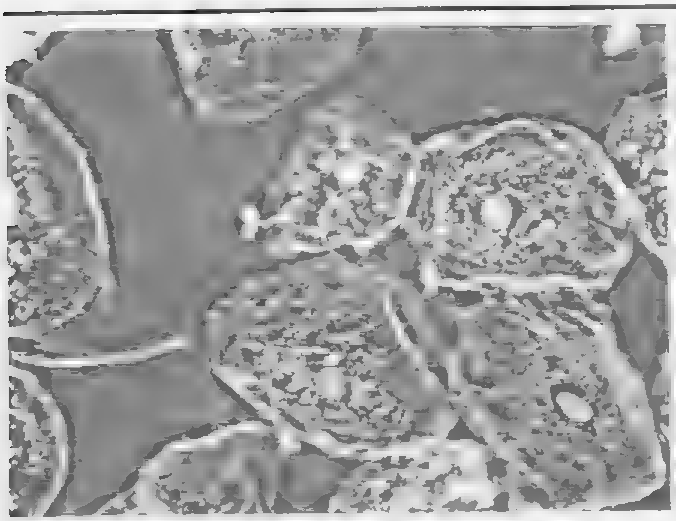
   NOTE: The top element of the standard working distance condenser should be approximately the thickness of a piece of paper beneath the underside of the slide. See "Preliminary Procedure" for stop screw adjustment.
8. Using the 10X objective, focus on the stained specimen.

   NOTE: If a separate illuminator is used with the 10X or 20X objective and the entire field is not fully and evenly illuminated, it is acceptable practice to use a ground glass filter. Neutral density filter(s) or clear color filters may also be inserted as required to reduce light intensity.
9. Close the field diaphragm, A *figure 21*, so that it is visibly superimposed on the specimen image. Focus the image of the field diaphragm at the same plane as the specimen by raising or lowering the condenser. Disregard field diaphragm centration at this point when using the 1036A In-Base Illuminator. After focusing, fully open the field diaphragm of the lamp.

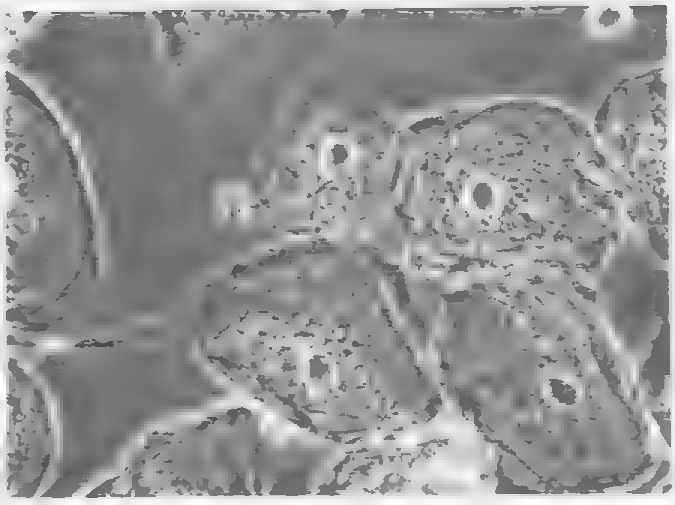
   NOTE: To facilitate focusing on the field diaphragm when using a separate illuminator, remove any ground glass or diffusing glass filters.
10. Leaving the phase turret set at "O", rotate the 45X phase objective into operating position. Using the fine adjustment knob, bring the image of the specimen into sharp focus.
11. Fully close the field diaphragm. Without disturbing the fine focus setting, bring the image of the field diaphragm into focus at the same plane as the specimen by raising or lowering the condenser by means of knob G *figure 20*.

    Use the aperture diaphragm of the condenser to enhance contrast of the field diaphragm as desired. Center the field diaphragm to the edge of the field of view by means of the condenser centering screws, E *figure 20*.

    NOTE: It is generally necessary when using a separate illuminator, to adjust the tilt of the plano mirror and the illuminator until the image of the field diaphragm is positioned in the field of view.



Epithelium Cells, Bright Contrast 100X Objective



Epithelium Cells, Dark Contrast 100X Objective

12. After centration of the field diaphragm, fully open the aperture diaphragm. All phase microscopy is carried out with the aperture diaphragm of the condenser fully opened.

    NOTE: Subsequent touch-up of the field diaphragm centration when changing objectives should be accomplished with centering screws B *figure 21* on the 1036A Illuminator.

13. To center the annular diaphragm of the Turret Condenser to the diffraction or phase plate of the objective, follow this procedure:

    (a) Rotate nosepiece to return the 10X objective to operating position (or lowest power phase objective).

    (b) Place an *unstained* specimen slide on the stage and open the field diaphragm until the iris leaves just *disappear* from the field of view. (Do not open excessively or spurious light will be detrimental to contrast.) Using the 10X objective, (or lowest power phase objective), bring the specimen into as good focus as possible.

    (c) Turn the knurled turret disc to the "A", 10X annulus, (or lowest power annulus) setting. Push in the two wrenches C, *figure 20*. By turning the wrenches slightly as they are inserted, you will feel them properly engage into the centering screws of the centering mechanism.

    (d) Swing in the Aperture Viewing Device with lever C *figure 22* (or remove eyepiece and insert Phase Telescope).

    (e) Bring the image of the annulus of the condenser and the diffraction plate of the objective into simultaneous, sharp focus by moving the focusing knob, B *figure 20*, of the Aperture Viewing Device in or out.

    (f) Adjust the annular diaphragm centering wrenches, C *figure 20*, until the annulus image is positioned concentric with, and is superimposed on, the diffraction plate as shown in *figure 24*.

    (g) Swing out Aperture Viewing Device (or remove Phase Telescope and replace ocular).

14. Bring the phase specimen into sharp focus. The microscope is now ready for use with the 10X objective (or lowest power phase objective).

15. Center the other annuli to their respective phase objectives. (Always remember to withdraw the centering wrenches, C *figure 20*, before the tur-

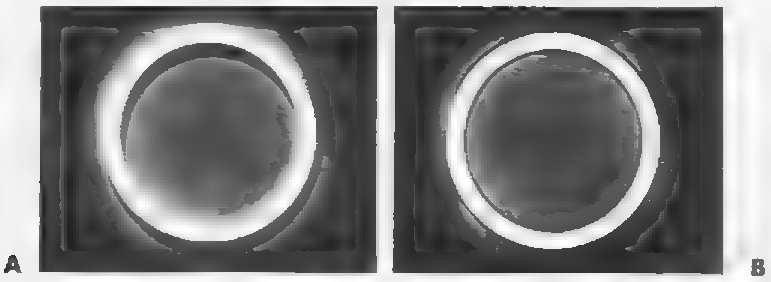
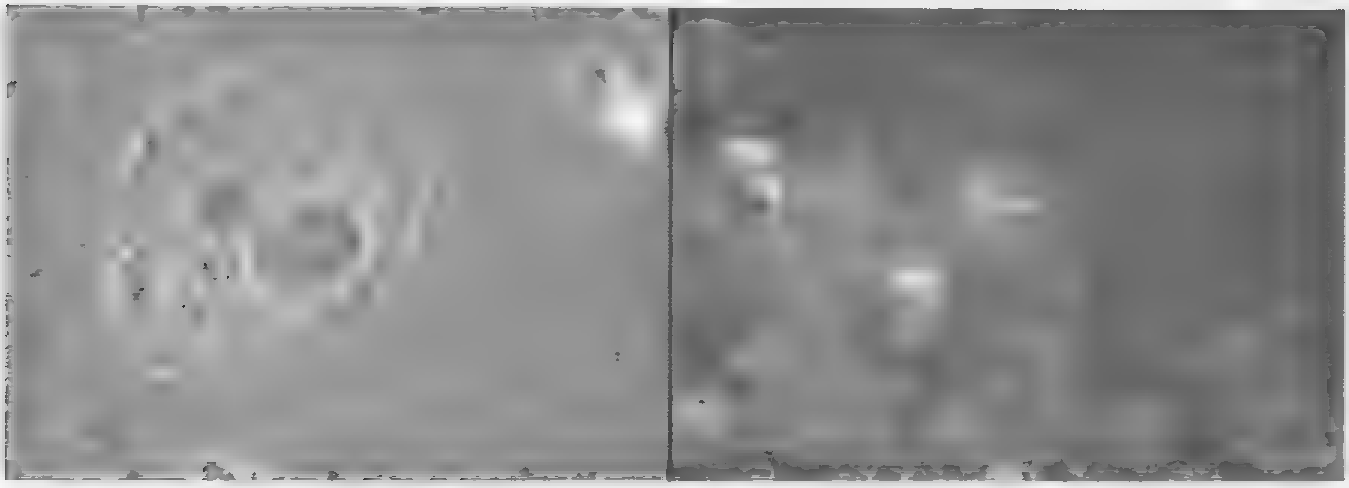


Figure 24 — Appearance of the image of the annulus and the diffraction plate of the objective when annulus is: (A) out of center; and (B) concentric with, and properly superimposed on, the diffraction or phase plate.



Oral Specimen, Dark Contrast 100X Objective

Oral Specimen, Dark Contrast 100X Objective

ret is rotated.) Once the annuli have been centered to a given set of objectives, they will remain centered for considerable periods of time with careful use of the microscope. Centration should be checked from time to time, particularly for critical observation or photomicrography.

16. In phase microscopy, when changing from one objective to the next, bear in mind the importance of: (a) field diaphragm centration; (b) adjusting field diaphragm so that the field of view is *just* filled with light; and (c) focusing field diaphragm at the same plane as the specimen. The image of the annulus always should be completely filled with white light. If the annulus image is partially colored, it is an indication of incorrect adjustment of condenser height.

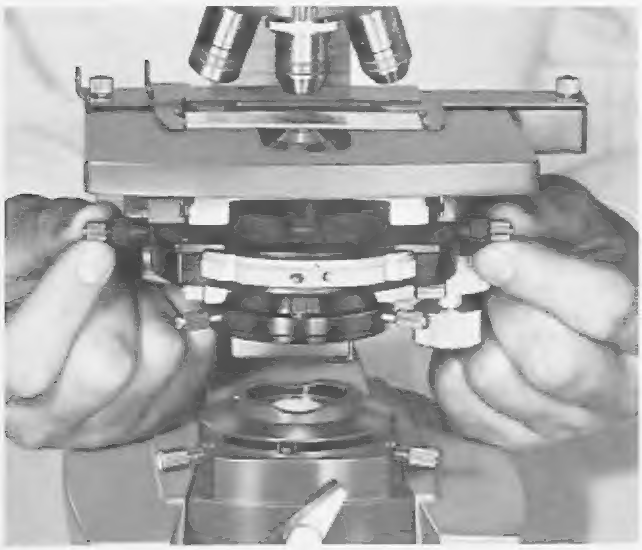


Figure 25 — To engage the annulus mount, push in the two centering wrenches and rotate slightly until you feel the allen head wrench ends move into secured position. When using the B-minus objective, without the diffraction plate visible, adjust wrenches until best contrast is achieved.

SPECIAL DIRECTIONS FOR B-MINUS OBJECTIVES

The low contrast B-minus objective has no absorbing materials on the diffraction plate and consequently cannot readily be seen with the Aperture Viewing Unit or Telescope. Centration of the annular diaphragm in the condenser, therefore, must be accomplished in a somewhat different manner.

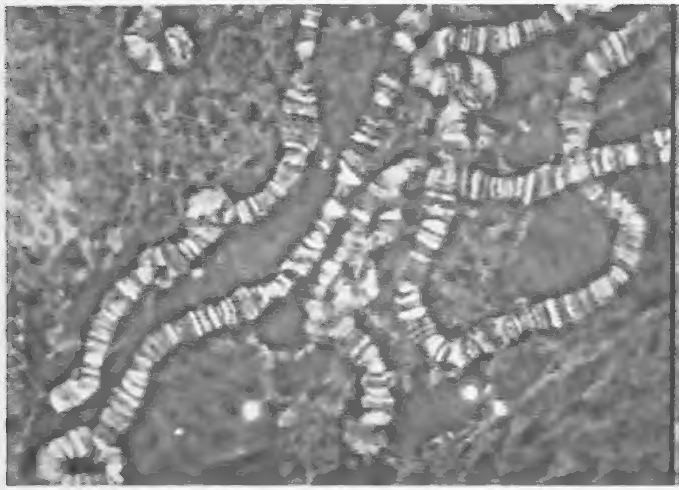
For best results, first use a 100X Dark or Bright Contrast phase objective and center the annulus with respect to this objective following the steps in "Optical Alignment Procedure" beginning on page 8. After proper centration is accomplished and the specimen is in sharp focus now rotate the nosepiece to the B-minus objective. Slightly turn the two captive centering wrenches, C *figure 20* used to align the condenser annuli and adjust until best contrast is obtained.

GENERAL COMMENTS ON TECHNIQUE

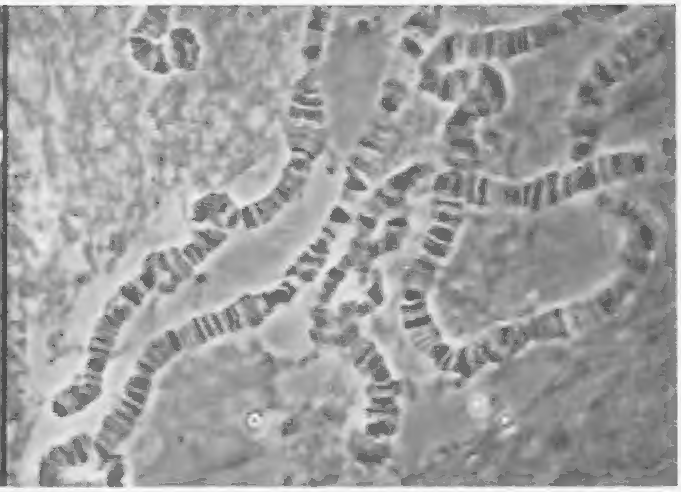
A few comments on technique are offered here as guidelines in the correct orientation of other annuli to their respective phase objectives.

When using the 45X objective, in some instances the specimen (i.e. a tissue section) may cause light diffusion to a degree which makes focusing the field diaphragm at the plane of the specimen difficult. In such cases, move to a clear area of the slide to accomplish the above; however, do not change the fine focus adjustment of the microscope when using this procedure.

When centering the field diaphragm using the 100X objective, fully close the lamp iris. Locate the east-west boundaries of the area of illumination by alternately moving them into the field of view by adjusting mirror tilt with a separate illuminator or by using the centering screws of the 1036A In-Base Illuminator. When so oriented to the positions of the light bound-



Drosophila Chromosomes, Bright Contrast 100X Objective



Drosophila Chromosomes, Dark Contrast 100X Objective

aries, arbitrarily select the center-most position of the area of illumination.

In adjusting condenser height to bring the field diaphragm into proper focus at the same plane as the specimen, the field of view should be free of color (with perhaps a suggestion of gray only). The presence of color is particularly significant when utilizing the 100X oil immersion objective. A bluish colored area seen in the field of view indicates that the microscope condenser is focused too high. A brownish-orange color indicates that the condenser is adjusted too low. Other color aberration is usually indicative of improper lamp, mirror or condenser centration.

The use of plano-plano specimen preparations is essential to clear imagery of the annular diaphragm to the diffraction or phase plate. *Figure 26* illustrates an example of distortion caused by a wedge shaped preparation. When such a "cat's eye" or other effect occurs, suspect first that your specimen preparation is not plano-plano. Check by turning the slide or mount 180°. If the distortion of imagery is reversed, this is evidence of irregularity in the preparation.

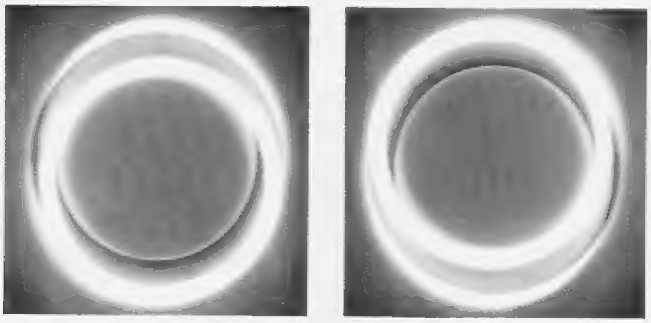


Figure 26 — Turn slide end-for-end when you suspect specimen preparation is not plano-plano. When imagery distortion is reversed, it is evidence of an irregularity in the specimen preparation, such as a wedge shaped condition.

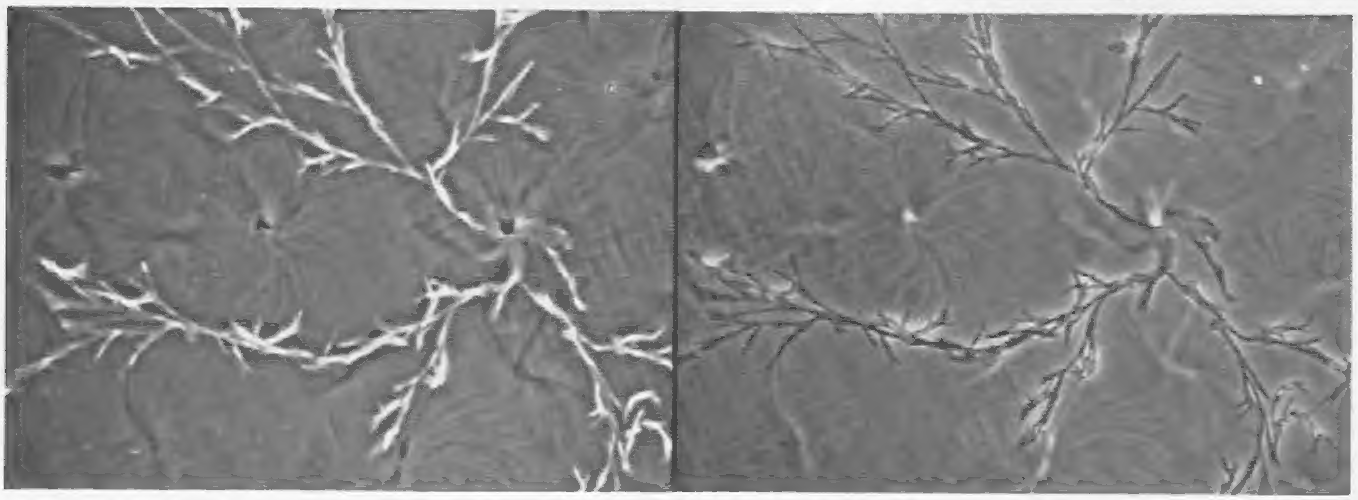
THE USE OF INTERMEDIATE AND LONG WORKING DISTANCE PHASE EQUIPMENT

The working distance provided with AO intermediate phase equipment is to 3mm in air (or the optical path equivalent in water or in crown glass). With the long working distance condenser, 15mm in air, or equivalent optical path, is provided. (See *figure 27.*) "Working distance" as defined here is the distance from the top of the microscope stage to the focal plane of the specimen. The interchangeability of AO phase equipment permits fast and easy modification of your PHASESTAR or AO *Series 10* Microscope from standard working distance usage to intermediate or long working distance.

Changing from standard to intermediate working distance merely requires the use of the No. 1243 Intermediate Working Distance Phase Condenser. Simply unscrew the No. 1242 Standard Condenser from the condenser mount (Turret or Single Condenser Mount) and screw the No. 1243 Condenser in place. This is the only change required as the same annuli are used for both working distances. All objective magnifications (10X, 20X, 45X and 100X), in the phase contrast desired, are utilized in intermediate working distance phase microscopy.

When changing from standard or intermediate to long working distance, the annuli (10X, 20X) in the Turret or Single Condenser Mount, in addition to the condenser, must be changed. To gain access to the annular diaphragm, remove the Standard or Intermediate Working Distance Condenser from the type of mount being used. Using the spanner wrench provided, unscrew the standard annulus and replace with the desired Long Working Distance Annular Diaphragm(s); then attach No. 1244 Long Working Distance Phase Condenser to Turret or Single Unit Mount.

The use of plano-plano specimen preparations is of paramount importance in phase microscopy. When utilizing intermediate and long working distances,



Resin, Bright Contrast 45X Objective

Resin, Dark Contrast 45X Objective

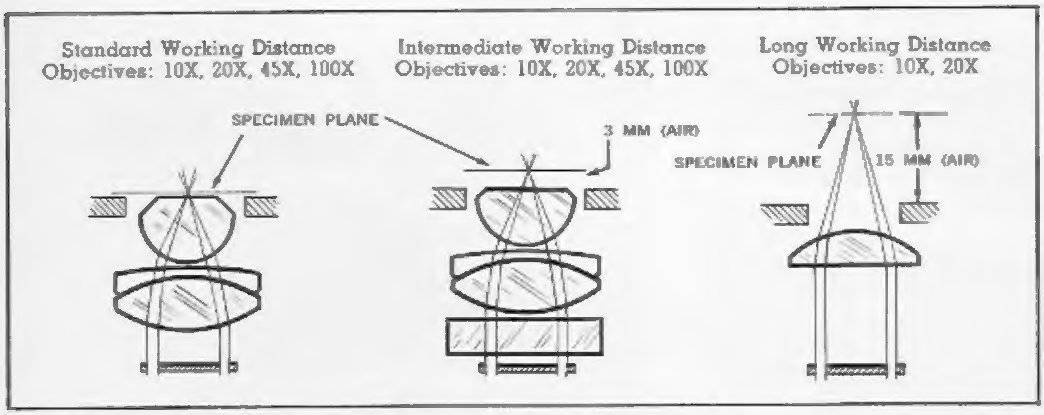


Figure 27 — One mm of air equals 1.33mm of water or 1.52mm of crown glass. (1mm of water equals 0.75mm of air. 1mm of crown glass equals 0.66mm of air.) These ratios of refractive indices can be used to obtain the equivalent working distance when the specimen includes more than one medium.

the specimen mount should have parallel walls with flat surfaces and be of good optical quality. Irregular, lens shaped (hollow ground slides) or wedge shaped preparations upset the alignment of the phase system. Even with only a small wedge, as when the cover glass is not quite parallel with the slide, it may be necessary to repeat centering procedures each time the specimen is moved to maintain good contrast. Phase microscopy is of little advantage with test tubes because of distortion of the annulus image making coincidence with the phase plate impossible. Cleanliness of specimen preparations is also most important to satisfactory intermediate and long working distance results.

The general set up procedure for intermediate and long working distance phase microscopy is the same as for standard distance. (See "Optical Alignment Procedure"); however, one additional step is suggested in the set up sequence. This follows Step *No. 13, e, page 10* (when the image of the annulus in the condenser mount is brought into sharp focus with the diffraction plate of the objective). At this point, adjust the condenser height slightly so as to obtain as good a "match" (same relative size) as possible between the image of the annulus and the diffraction plate as seen through the Aperture Viewing Unit or Telescope. After such adjustment for size, proceed with centration.

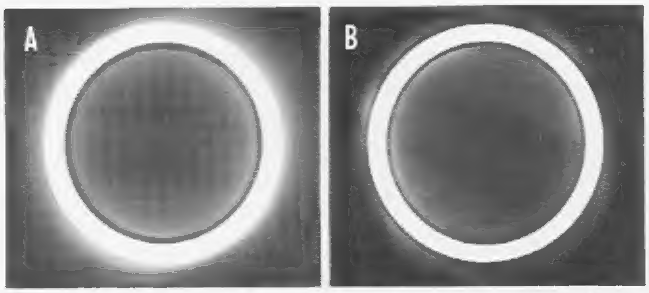


Figure 28 — In photograph (A), the image of the annulus is out of proportion in relative size to the diffraction plate. The image is too large and extends beyond the outside edge of the diffraction plate. By adjusting condenser height, a good "match" is obtained as shown in (B).

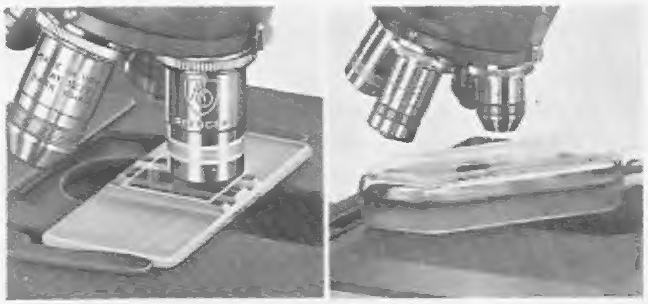
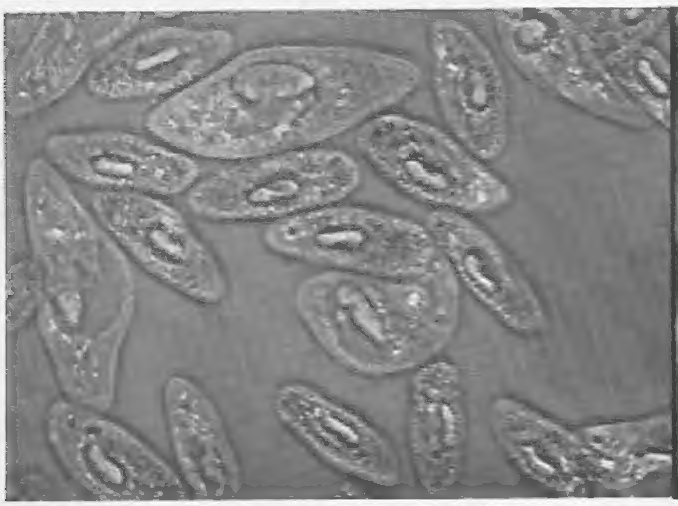
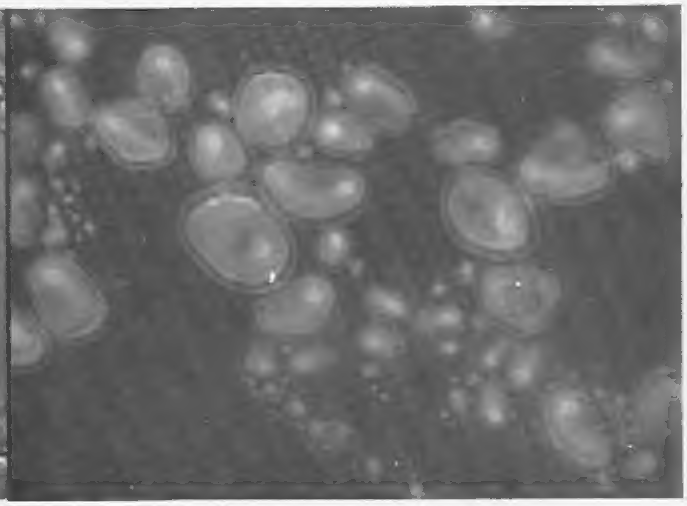


Figure 29 — Plano-plano specimen preparations are essential to good phase microscopy. Mounts should be flat, parallel and of good optical quality. Flat bottom Hemacytometers, AO No. 1475, are recommended for phase microscopy.



Paramecia Protozoa, Bright Contrast 10X Objective



Starch, Dark Contrast 20X Objective

OPERATION OF THE PHASE SINGLE CONDENSER MOUNT

Operation of the No. 1241 Condenser Mount is generally the same as that of the Turret Condenser. It accepts the Standard and Long Working Distance Phase Annuli which are attached or removed from the condenser with a spanner wrench *(figure 31)*. All three condensers, Standard, Intermediate and Long Working Distance, may be used with the Single Unit Mount.

After inserting the desired annuli and attaching condenser, direct the light from the illuminant into the condenser mount. Focus the filament image of the lamp at approximately the lower plane of the condenser annular diaphragm.

Centration of the condenser is not required with the Single Mount. When attaching the Mount to the fork of the microscope substage assembly, make certain that the pin in the fork engages or "snaps in" the slot in the mount and that the knurled holding screw is tightened.

Alignment of the annulus to the diffraction plate of the objective is the same as the Turret. The two captive centering wrenches of the mount, as shown in *figure 30*, permit fast and easy centration.

With the exceptions of the points above, follow the steps under "Optical Alignment Procedure."

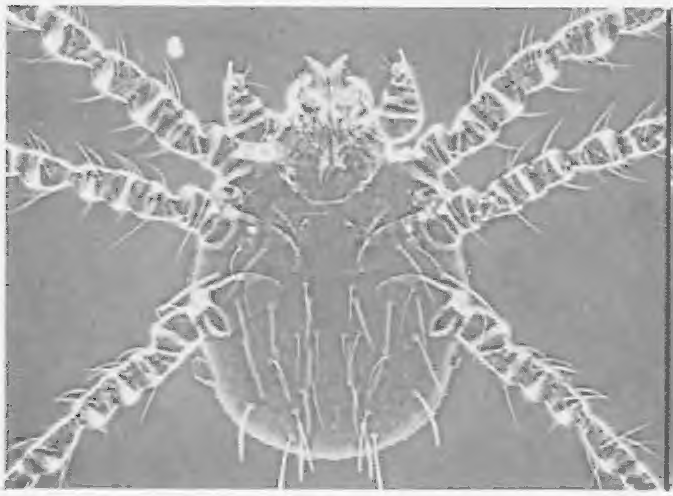
PHASE PHOTOMICROGRAPHY

Pictures with excellent detail and contrast can be taken with your PHASESTAR or *Series 10* Microscope with phase accessories. Exposures with B-minus Contrast will be about the same as with ordinary Brightfield; Dark and Bright Contrast usually require appreciably longer exposures (about 10 times). Make certain that the annulus and the phase plate of the objective are concentric with no evidence of spurious light when viewing through the Aperture Viewing Unit or Telescope.

Figure 30

Figure 31

Figure 32



Mite, Bright Contrast 20X Objective

Mite, Dark Contrast 20X Objective